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File: 71-047

FROM: J. C. Yang

DATE: January 28, 1976

SUBJECT: Procedure and Equipment for Fiber Counting

Attached is a detailed method and equipment list for fiber counting, written by F. G. Serafin.

If you have any specific areas requiring elaboration, please contact me or F. G. Serafin.

The time required for fiber counting (including calculation and cleaning) is about 30-45 minutes depending upon the skill of the operator.

Julie C. Yang

JCY:mlr

attachment

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I. EQUIPMENT NEEDED FOR SLIDE PREPARATION

1. Mounting medium - see Appendix for preparation.

2. Ross Optical Lens Tissue - this lens tissue is preferred because of its superior cleaning qualities. It may be purchased from Ladd Research Industries, P.O. Box 901, Burlington, Vermont 05401. A dozen boxes at 1000 sheets each should last some time.

3. 10 gross of 75 x 25 mm precleaned glass slides with one end frosted. Fisher Scientific supplies these as item 12-552.

4. 1 case of 22 x 22 mm no. 1.5 (0.16 to 0.19 mm thick) cover glasses. Fisher item 12-541B.

5. 2 curved forceps. Fisher item 8-875.

6. 2 dissecting knife handles. Fisher item 8-913.

7. 6 dissecting knife blades. Fisher item 8-916D.

8. 1 package of 125 mm x 3 mm stirring rods. Fisher item 11-380A.

9. Pencil.

10. Small spatula or knife (aside from the one used to cut the filter paper) to cut the tape around the cassette.

11. Quarter or nickel for prying open cassette.

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II. SLIDE PREPARATION

1. Place clean lens tissue on desk.

2. Wipe dissecting knife, forceps, and 1 stirring rod end with another piece of lens tissue using a separate section of the tissue for each instrument. After wiping each instrument, it should be placed on the tissue in step (1). The wiping tissue should be thrown away.

3. Place a second piece of clean lens tissue on the desk.

4. Take hold of a glass slide by its edges at the frosted end and hold it up to the light. Remove any visible dirt by wiping with lens tissue. Put the slide down on the clean tissue in step (3) frosted side up, and replace the cover on the box of remaining slides.

5. Take hold of a cover glass by its edges, hold it up to the light checking for cleanliness and, again, remove any visible dirt by wiping with lens tissue. Place it on the clear edge of the glass slide so that it overhangs enough so that one of its edges is resting on the lens paper. Close the cover on the remaining cover glasses.

6. Cut the sealing tape on the air sample cassette with a spatula, knife, or fingernail.

7. Loosen the cassette by prying around the edges with a coin (nickel or quarter should do) but do not remove the cover yet.

8. Place a drop of mounting medium (see Appendix for preparation) in the center of the slide. This should be done with the stirring rod which comes with the Balsam bottle. The first drop should be permitted to fall back into the bottle; the second drop should fall on the slide (never touch the slide with this rod). Always place the cover on the bottle after you are finished putting the drop on the slide.

9. Pull one edge of the drop of mounting fluid,on the slide, out so as to get a pear shaped drop. This is done with the clean stirring rod on the tissue from step (2). After doing this, the stirring rod should be wiped dry with the center of another piece of lens tissue and replaced in its original position. Don't discard the cleaning tissue yet.

10. Remove the top of the sample cassette completely.

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11. Holding the cassette containing the filter paper firmly on the desk with one hand, cut a pie shaped segment from the filter paper with an arc of about one cm. using the dissecting knife. The cuts should be made starting from the filter paper's center and extending out to its edge. Wipe both sides of the scalpel blade carefully with a clean portion of the paper used to wipe the rod in step (9) and replace the knife in its original position. (Wipe the blade pulling the cutting edge toward yourself rather than pushing it toward you. The latter technique results in rather bloody cuts.) Again, don't discard the cleaning tissue.

12. Remove the pie shaped filter segment from the cassette using the curved forceps and place it on the drop of mounting medium so that the drop and segment shapes correspond. The paper should be grasped by its circumferential edge with the forceps, to minimize disturbing the dust on the paper's surface. Replace the cassette cover.

13. Place the cover glass over the sample. Wipe the forceps clean with a clean portion of the paper from step (11), and now discard the latter. Apply a gentle pressure on the cover glass using the convex curve of the forceps for about 30 seconds. Then put the forceps back in their original position.

14. On the frosted end of the slide write in pencil, using a three-line format, giving sample identification, date of slide preparation, and your initials. Put the slide aside on a piece of lens tissue.

15. Repeat the pressure described in step (13) two more times - once after each of the next two slide preparations. This should clear the central portion of the filter paper leaving only the three corners of the original pie visible if the size of the mounting medium drop was correct.

16. A new lens tissue should be set out for the preparation of each new slide (step 3).

17. The slide is ready for reading 0.5 hour after preparation. But waiting for 0.5 to 1 day is better, because there is some early graininess to a fresh slide which makes it more difficult to read. Slides should be read within 5 days after preparation to prevent any difficulties from possible crystallization of the mounting medium.

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III. MICROSCOPE PRELIMINARIES

This section is written in general terms since the procedures are dependent on the make and model of the microscope used.

The Porton reticle should be calibrated against a slide micrometer. Ours has been adjusted to 50 microns to a side, resulting in a counting field area of 0.0025 mm^2.

Place a sample slide on the stage as for counting. Then:

1. Focus on some particulates on the filter paper area of the slide.
2. Adjust the light source for maximum brightness.
3. Examine the phase rings for correct overlap. Adjust if necessary.
4. Close the illuminator iris and adjust the substage condenser until the image of the iris opening is sharp and on the verge of changing colors (blue to red or vice versa).
5. Open the illuminator iris until the field of view is just filled with light.
6. Adjust the fine focus to get the best image.

I have found that a single check of the phase rings per day is sufficient unless the objectives are changed to some other power. This usually disrupts ring alignment in our scope (as do the vibrations of a passing freight train) and step 3 above must be repeated. Adjustment of the substage condenser is more variable and can easily be checked for each slide with our microscope.

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IV. COUNTING

After the preliminaries of section III have been completed, the slide is ready to be counted. We count 40 fields. However, if time is available, more fields should be counted to get a statistically more reliable figure, especially if the fiber counts are low. The 40 fields are selected at random in four sweeps across the filter paper area with ten stops during each sweep. We stay away as best we can from the edges, the apex, and the arc of the original pie shaped segment.

Only those fibers in the left hand square of the Porton reticle are counted. This square constitutes the counting field. A decision should be made prior to counting concerning fibers which cross the boundaries of the square field. Two adjacent sides of the square should be chosen so that fibers crossing those sides are counted while fibers crossing the other two sides are ignored even if a portion of them lies within the field.

Only those fibers greater than 5 microns in length are counted. Lengths may be estimated by comparison with the hash marks along the left side of the Porton field square. (Based on our calibration, the distance between the closest marks in our microscope is conveniently set at 5 microns.) The circles along the bottom edge of reticle may also be used. Only those fibers whose length to diameter ratio (aspect ratio) is greater than 3 should be counted. Do not count fibers whose diameter is greater than 3 microns, and do not count as fibers crystals or slivers of material. Crystals can be particularly deceptive if an edge is visible and the refractive index of the crystal is a near match for that of the mounting medium (a good example of this problem is perlite).

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V. CALCULATION OF RESULTS

The calculation of the number of fibers/ml. of air samples is done as follows:

(1) the sampling area of Millipore filter paper AAWP03700 is 855 mm^2 (we have checked this and found it to be correct). However, you should doublecheck this to make sure for your filter paper.

(2) divide the total sampling surface area of the filter paper (855 mm^2) by the area of one counting field to get the total number of possible fields the filter paper may be divided into (in our case this is 855 ÷ .0025 = 342,000).

(3) multiply the result in step (2) by the average number of fibers found per field to get the total number of fibers on the whole filter disc.

(4) multiply the total time air was sampled by the pump (in minutes) by the flow rate through the pump (in ml./minute).

(5) divide the result in (3) by the result of (4) to get the final answer in terms of fibers per ml. of air.

These calculations are summarized by the following formula and example.

$$\text{fibers per ml. of air} = \frac{K \times F}{V \times T}$$

where, T = sampling time in minutes
V = air flow rate through pump in ml. per min.
F = average number of fibers per field
K = total sampling filter disc surface area divided by area within one counting field (both surface areas should be calculated using the same units, i.e., cm^2 or mm^2, etc., and only the actual sampling area of the filter paper should be included and not that covered by the upper plastic rim of the cassette).

Example:

T = 15 minutes
V = 1.5 liters/min. or 1500 ml./min.
F = 6 fibers/40 fields = 0.15 fiber/field
K = 855 mm^2 ÷ .0025 mm^2 = 342,000

$$\frac{342{,}000 \times 0.15}{1500 \times 15} = 2.28 \text{ fibers per ml. of air}$$

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APPENDIX

1. Add 15 ml. of dimethyl phthalate to 15 ml. of diethyl oxalate and stir thoroughly to get complete mixing (use very clean equipment throughout to avoid contamination).

2. Weigh out 1 g. of Millipore AAWP03700 filters (these are the same as the filters used to collect the dust samples) and place these in a Balsam bottle (Fisher Cat. No. 3-082).

3. Add 20 ml. of the solution prepared in Step (1) to the Balsam bottle and stir the contents briefly. Repeat the brief agitation once a day for about three or four days by which time the filter paper will have dissolved leaving a slightly cloudy solution ready for use as the mounting medium.

4. Always cover the Balsam bottle when not preparing or using its contents.

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